# Phytotoxic metabolites from *Neofusicoccum parvum*, a pathogen of Botryosphaeria dieback of grapevine

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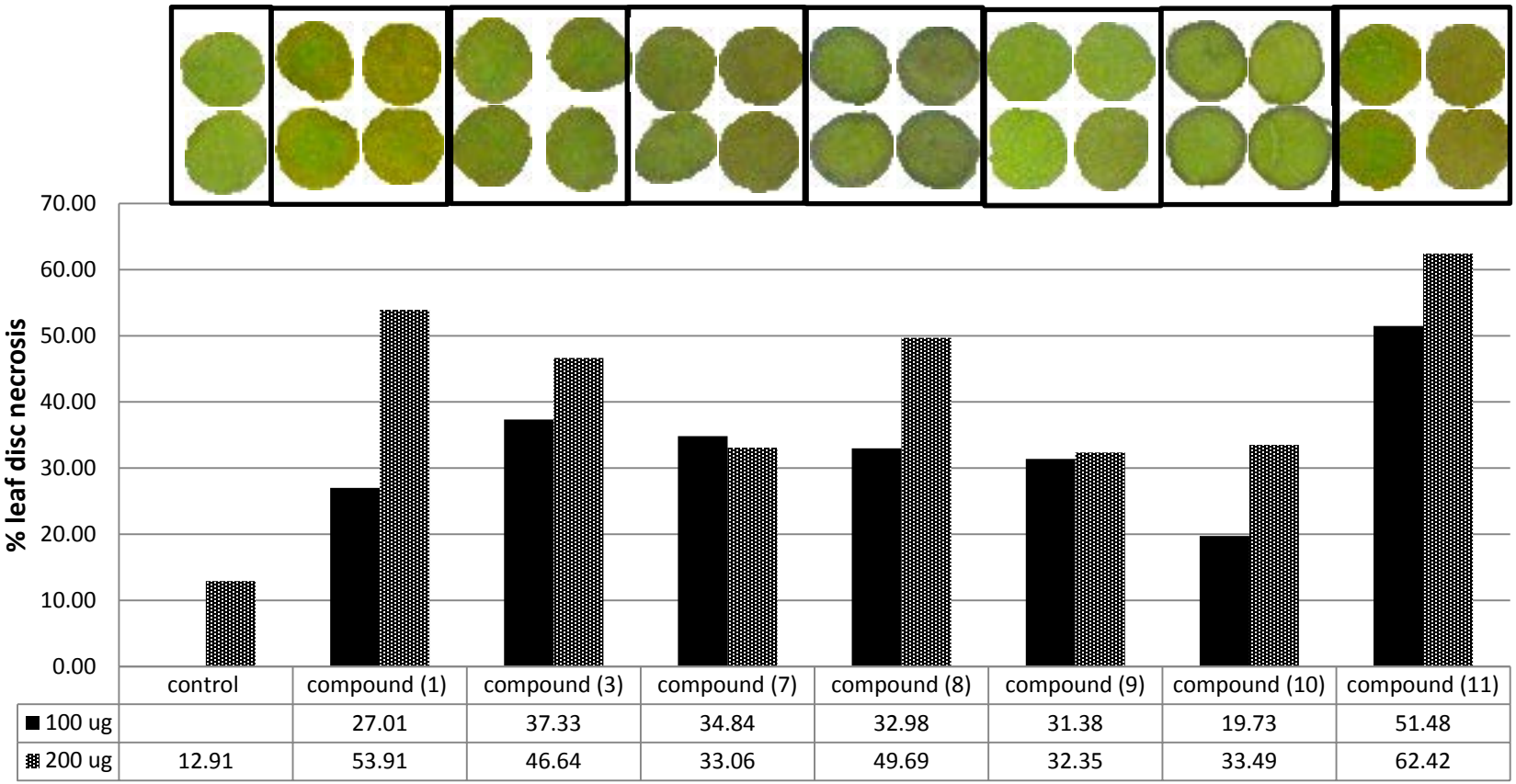


| | control | compound (1) | compound (3) | compound (7) | compound (8) | compound (9) | compound (10) | compound (11) |
|---|---|---|---|---|---|---|---|---|
| 100 ug | | 27.01 | 37.33 | 34.84 | 32.98 | 31.38 | 19.73 | 51.48 |
| 200 ug | 12.91 | 53.91 | 46.64 | 33.06 | 49.69 | 32.35 | 33.49 | 62.42 |

**Figure 2: Necrosis induced by seven of the isolated phytotoxins at concentrations of 100 and 200 µg.mL$^{-1}$ 48 h post inoculation.** Quantification of necrosis in leaves of *V. vinifera* cv. Chardonnay after 48 h (n = 3; ±SD). The experiment was repeated twice with similar results. (-)-terremutin (**1**); (+)-*epi*-sphaeropsidon (**3**); (+)-(6*R*,7*S*)-dia-asperlin (**7**); (-)-mellein (**8**); (-)-(3*R*,4S)-*trans*-4-hydroxymellein (**9**); (-)-(3*R*,4*R*)-*cis*-4-hydroxymellein (**10**); (-)-(*R*)-3-hydroxymellein (**11**).

A representative picture of the necrosis caused by the toxin is placed above each histogram as a visual illustration.

| Sample | % of leaf necrosis | Average 100 ug.mL-1 | % of leaf necrosis | Average 200 ug.mL-1 | STD 100ug.mL-1 | STD 200 ug.mL-1 |
|---|---|---|---|---|---|---|
| control | | | 20.588 | 12.91 | 0 | 6.3 |
| | | | 11.497 | | | |
| | | | 6.653 | | | |
| compound (1) | 20.7 | 33.2 | 51.04 | 55.4 | 5.6 | 4.3 |
| | 27.07 | | 59.47 | | | |
| | 33.25 | | 51.23 | | | |
| compound (3) | 35.197 | 31.1 | 46.667 | 46.64 | 6.7 | 4.8 |
| | 31.161 | | 41.281 | | | |
| | 45.644 | | 51.985 | | | |
| compound (7) | 36.19 | 34.84 | 52.321 | 33.06 | 6.2 | 2.5 |
| | 27.377 | | 48.295 | | | |
| | 40.952 | | 53.73 | | | |
| compound (8) | 35.133 | 32.98 | 49.908 | 48.5 | 2.8 | 1 |
| | 29.412 | | 50.625 | | | |
| | 34.381 | | 48.529 | | | |
| compound (9) | 32.782 | 31.38 | 29.309 | 32.35 | 1.9 | 4.7 |
| | 28.966 | | 29.323 | | | |
| | 32.386 | | 38.408 | | | |
| compound (10) | 19.301 | 19.73 | 31.913 | 33.49 | 3.1 | 4.4 |
| | 23.363 | | 39.02 | | | |
| | 16.532 | | 29.529 | | | |
| compound (11) | 58.712 | 51.48 | 63.971 | 62.42 | 6.8 | 6 |
| | 52.139 | | 68.199 | | | |
| | 43.592 | | 55.089 | | | |

**Figure 2: Necrosis induced by seven of the isolated phytotoxins at concentrations of 100 and 200 μg.mL$^{-1}$ 48 h post inoculation.**

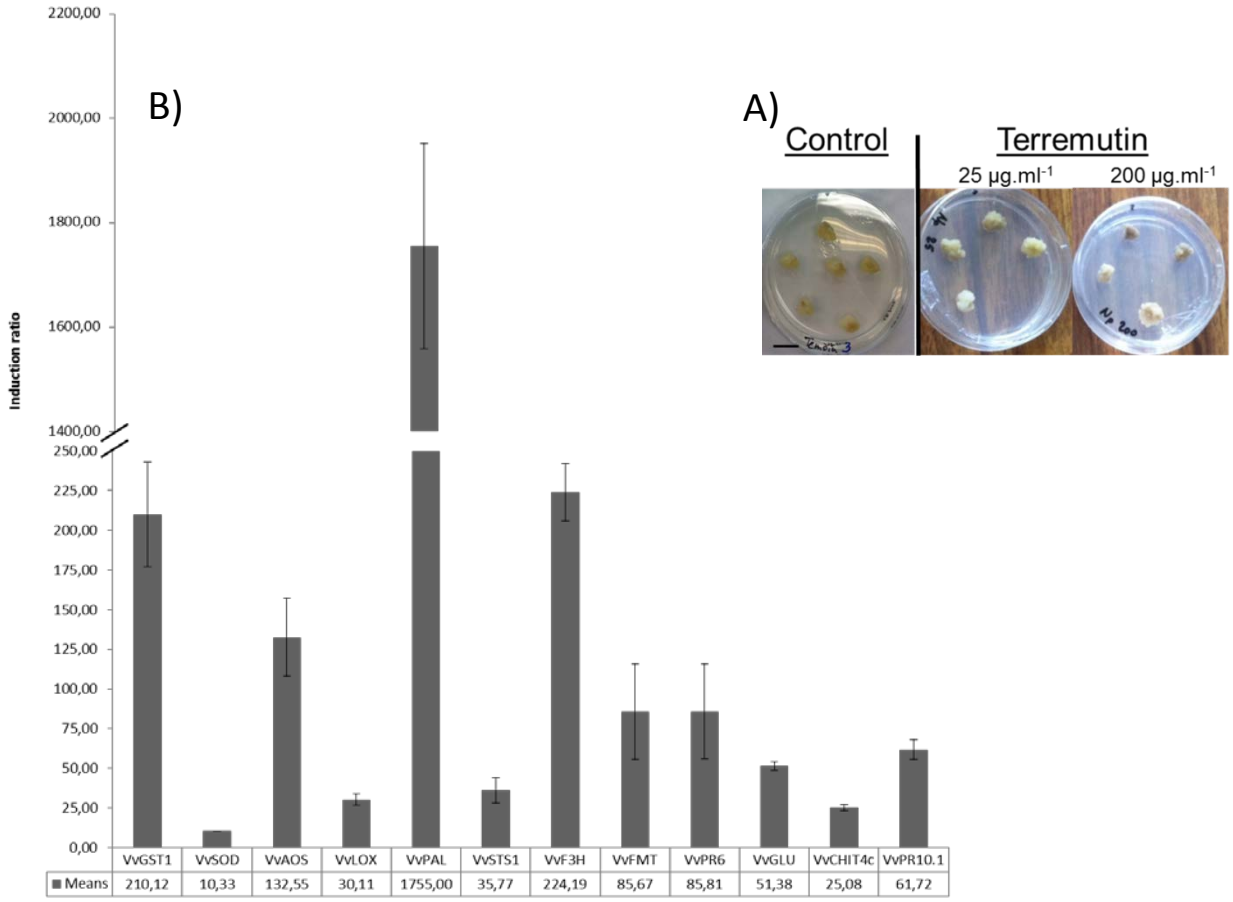


**Figure 3: A) Calli subcultured in Petri dishes with malt medium:** (control) and (-)-terremutin (**1**) (25 and 200 µg.mL$^{-1}$) 6 days post inoculation. B**) Defence gene expression in calli of *V. vinifera* cv. Chardonnay subcultured in Petri dishes with 200 µg·mL$^{-1}$ terremutin (1) at 6 days post inoculation**. Gene expression was assessed by RT-qPCR, and the values shown are relative to the non-induced control samples. The genes tested encode cellular detoxification enzymes (*VvGST1* and *VvSOD*), jasmonic acid pathway enzymes (*VvAOS* and *VvLOX*), enzymes for secondary metabolites of the phenylpropanoid pathway (*VvPA*L), phytoalexin pathway enzymes (*VvSTS1*), flavonoid pathway enzymes (*VvF3H* and *VvFMT*), and pathogenesis-related proteins (*VvPR6*, *VvGLU*, *VvCHIT4*c, and *VvPR10.1*). Each data point represents the mean from three technical and two biological replicates. The error bars represent the standard errors of the mean. Significant differential gene expression was defined with an induction ratio higher than 4.